OFAS

Life's blood flows through the hourglass; the stopcock represents the alteration of aging and disease as biomedical research progresses.

Report of the Director

Dear Friends,

The Orentreich Foundation for the Advancement of Science is dedicated to studying ways to increase human healthspan and lifespan. Our scientists have become internationally recognized experts in understanding the role of reduced intake of sulfur amino acids (SAAR) in extending life expectancy and slowing the onset of many age-related diseases. Our research ranges from molecular biology to whole animal (including human) physiology. Recent work described in this report includes SAAR-related slowing of symptom onset in amyotrophic lateral sclerosis (ALS, or Lou Gehrig's Disease), changing protein structure and processing, and even developing novel dietary and non-dietary methods to achieve the SAAR effect.

In September, OFAS co-sponsored with the National Institute on Aging a workshop entitled "Optimizing dietary amino acid intake to improve human health and reduce the burden of age-related disease." This workshop (which marked the first time the NIA has co-sponsored a workshop with a private foundation) brought together experts in amino acid nutrition and aging to discuss the potential of specific amino acid changes in the diet to produce improved human healthspan and lifespan. NIA staff use workshops like this to identify areas of priority for future research funding.

OFAS continues to robustly support our investigators' research directly through Foundation funds, but outside support is always encouraged from donors or research grants. In this vein, we are very proud to announce that one of our investigators, Dr. Sailendra Nichenametla, has received a substantial 3-year research grant from the Hevolution Foundation to support his investigations into the mechanism of sulfur amino acid restriction in lifespan extension.

On behalf of the entire staff at OFAS we thank you for your support, faith, and interest in our mission. We wish you a healthy and happy 2024.

David S. Orentreich, MD, FAAD
Director

OFAS-NIA Joint Workshop on Healthy Aging

Ten years after our first Symposium on Healthy Aging, OFAS hosted a joint workshop with the National Institute of Aging. The two-day symposium began with the mechanisms of aging-related signal pathways in yeast and cell cultures which are conserved across taxa, and ended with translational applications of dietary- and pharmacological-interventions in humans. Below are session highlights; videos of most talks are available online at https://www.orentreich.org/ofas-nia-speakers/.

Mechanistic Studies of Healthspan-Extending Interventions in Cultured Cells

Jeffrey Smith, Ph.D.
University of Virginia School of Medicine
As a unicellular organism, the impact of the environment on budding yeast survival is expected to occur through cell-autonomous mechanisms such as direct changes in gene expression. However, regulation of survival in yeast is also linked with cell-extrinsic mechanisms. For example, media from yeast in caloric restriction (CR) extends the chronological lifespan of yeast not in CR. Using a met15Δ similar to mammals, to whom methionine is also essential, allows the lab to investigate pathways with the potential to contain therapeutic targets.

Christian Sell, Ph.D.
Drexel University College of Medicine
Cellular senescence, characterized by growth arrest and high glycolytic activity, is a cell fate in both somatic cells and stem cells. While senescence is conserved as a mechanism to limit the risk of oncogenic transformation, inflammation associated with senescence causes damage. Treatment with the mTOR inhibitor rapamycin can delay senescence; one mechanism may be via increasing levels of non-coding RNA molecule H19. H19 is thought to control gene expression by acting as a microRNA sponge, or by leading protein complexes to genes at the chromosome level.

Emerging Concepts in Dietary Restriction and Healthspan

Stephen Simpson, Ph.D.
University of Sydney
Because of the need for nitrogen, a protein-specific appetite exists in living organisms from insects to humans. While protein hunger appears unrelated to fat, carbohydrate, and total energy intake, the converse is not true. This means that when dietary protein is diluted by fat and carbohydrates, the strong protein appetite leads to excessive energy intake and obesity in the attempt to fill protein needs. Paradoxically, lifespan increases on a low-protein, high-carbohydrate diet. Dietary choices change depending on the goal and age: increased lifespan, increased fertility, and increased time of fertility determine nitrogen requirements.

Cara Green, Ph.D.
University of Wisconsin-Madison
While at a population level a low protein (LP) diet promotes weight loss, improves glycemic control, and increases lifespan, genetic analysis of 40 different recombinant inbred strains of male and female BXD mice found that the metabolic response to an identical LP diet varied by both strain and sex. Whether the same individual response will be found in humans will be important as dietary healthspan interventions move from the lab to the home.

Holly Brown-Borg, Ph.D.
University of North Dakota School of Medicine
Growth hormone (GH) is a pituitary-derived hormone that has both somatic and metabolic functions. Many similarities exist between reduced levels of GH and dietary MetR. Both interventions improve metabolic health and reduce oxidative stress, damage, and some indices of mitochondrial function and stress resistance, each of which contributes to the observed extensions of healthspan and lifespan. Despite the overlap, there are additive differences. The mechanisms underlying these responses and their relative contribution to the physiology of aging are complex and require further study.

Mechanistic Studies of Healthspan-Extending Interventions in Rodents

Sailendra Nichenametla, Ph.D.
OFAS
Caloric restriction (CR) and sulfur amino acid restriction (SAAR) are the two most successful dietary interventions that extend lifespan in laboratory models. While both induce similar health benefits, the underlying mechanisms are likely very different since CR alters the quantity of dietary intake while SAAR alters the quality, i.e., dietary composition. Comparing the effects of these two dietary regimens at the molecular level contributes to a better understanding of the basic mechanisms involved in aging and age-related diseases. Preliminary data from 8-week-old male rats on CR and SAAR diets indicate that these diets exert differential effects at the subcellular level.

Jay Johnson, Ph.D.
OFAS
Methionine restriction (MR) is one of only a few dietary manipulations known to dramatically extend mammalian healthspan. While technically feasible for humans, long-term adherence to such a regimen is likely to be challenging (or even undesirable) for many. An intermittent version of MR (IMR) not only has a much shorter interventional period than continuous MR (i.e., only 3 days per week of reduced methionine intake), but it also preserves bone mass and dramatically reduces the accumulation of marrow fat. In a complementary approach, we sought to identify compounds that produce MR-like healthspan benefits, but in a normal, methionine-replete context. Supplementation with any one of four different amino acids is sufficient to produce the same metabolic health benefits typically observed for MR. Specifically, animals supplemented with these amino acids are completely protected against diet-induced obesity and demonstrate similar beneficial plasma hormone changes as continuously methionine-restricted mice.

Advances and Challenges in Translating Dietary and Pharmacological Interventions

Matt Kaeberlein, Ph.D.
Optispan Inc. and University of Washington
Some physicians prescribe rapamycin off-label as a preventative therapy to maintain healthspan. However, there is limited data on side effects or efficacy associated for it used in this context. To begin to address this gap in knowledge, adults with a history of off-label rapamycin use were surveyed. The only condition significantly more common in rapamycin users was presence of mouth ulceration, a known rapamycin side effect. Several conditions were significantly less frequent in rapamycin users: abdominal cramps, depression, muscle tightness, anxiety, and others.

Raghu Sinha, Ph.D.
Penn State College of Medicine
Penn State and OFAS researchers collaborated on the first randomized, controlled-feeding study in humans examining both methionine-restricted (MR) and methionine- and cysteine-restricted (SAAR) diets in healthy adults. No adverse effects were seen with either at either level of restriction studied. SAAR was associated with significant body weight reduction; it induced beneficial changes in biomarkers of cardiometabolic disease risk. Fewer changes were seen in MR. This indicates that SAAR without calorie restriction is a feasible, safe, and effective intervention. These beneficial changes occurred in as little as 4 weeks after initiation of the restricted diets and occurred similarly in both men and women.

Thomas Olsen, Ph.D.
University of Oslo
Men and women aged 18-45, overweight but otherwise healthy, were randomized to a diet with either low- or high-sulfur amino acid (SAA) content for 8 weeks. Both groups ate a base diet of low-SAA plant-based whole foods. SAA content was provided via capsules containing either carbohydrate or a powdered methionine and cysteine supplement. This allowed a double-blind intervention. The low-SAA group had 20% greater weight loss with no significant change in energy expenditure versus the high-SAA cohort. Some benefits (e.g., decreased serum leptin) were similar to that seen in SAAR animal models; others (e.g., fasting glucose) were not.

Nichenametla Laboratory

Lipid metabolism, our ability to synthesize, store, and utilize body fat, worsens with aging. This worsening leads to obesity, diabetes, and metabolic syndrome, which in turn increase the risk for cancers and heart diseases. Treating impaired lipid metabolism is beneficial in multiple diseases.

Sulfur amino acid restriction (SAAR)—decreasing the dietary concentration of the sulfur amino acids methionine and cysteine—exerts strong anti-obesity effects in rats and mice. However, formulating the SAAR diet for human consumption is very difficult. Clinical studies conducted with the best-possible versions of the SAAR diet observed only modest benefits. Dr. Nichenametla's laboratory focuses on understanding the anti-obesity mechanisms of the SAAR diet in rodents and inducing them with drugs in humans. Their recent data strongly suggest that SAAR diverts the biochemical precursors required for fat synthesis (glyceroneogenesis) to synthesize an amino acid called serine (serinogenesis) (*Aging Cell*, 2022; 21(12): e13739). This data is consistent with their findings from an epidemiological study in that individuals with high plasma serine had lower plasma triglycerides and lower incidence of metabolic syndrome.

Dr. Nichenametla hypothesizes that increasing serinogenesis induces a lean phenotype by depleting precursors from glyceroneogenesis. Using two independent approaches, his lab will confirm whether glyceroneogenesis decreases exclusively due to serinogenesis. In the first approach, they will use genetically modified mice to inhibit serinogenesis while feeding on the SAAR diet; failure of the SAAR diet to induce fat loss in these mice will confirm serinogenesis as the underlying mechanism. In the second approach, they will inject mice with stable isotopes (tagged nutrients) and track the percent of biochemical intermediates they use for serinogenesis as opposed to glyceroneogenesis. A higher utilization of tagged nutrients is expected for serinogenesis.

Further experiments will test if serinogenesis induces any beneficial effects unrelated to glyceroneogenesis. Serine alters the levels of acylcarnitines, sphingolipids, and ceramides, all of which affect lipid metabolism. To find if serinogenesis promotes a healthy lipid profile (increased concentrations of good fats), Dr. Nichenametla's team will conduct lipidomics analysis, which identifies relative proportions of all different types of fats in the livers.

Finally, the group will investigate whether serinogenesis is druggable. Since low plasma levels of cysteine are associated with a lean phenotype, they will treat mice with two cysteine-depleting drugs, 2-mercaptoethane sodium sulfonate (Mesna) and DL-buthionine sulfoximine (BSO), to determine if they increase serinogenesis and exert effects on lipid metabolism comparable to those of the SAAR diet. Mesna increases the urinary excretion of cysteine and BSO inhibits the synthesis of glutathione, the storage form of cysteine. The laboratory will also quantify the plasma levels of serine, cysteine, and triglycerides in humans treated with Mesna.

Sailendra Nichenametla, Ph.D.
Senior Scientist

Overall, the research will shed light on the role of serinogenesis in lipid metabolism and whether it can be targeted to treat human diseases. The data will be applicable not only to lipid metabolism disorders but also to other diseases such as Alzheimer's disease, cancers, and macular degeneration, in which serine plays a crucial role.

Dr. Nichenametla is an inaugural recipient of the Hevolution/AFAR New Investigator Awards in Aging Biology and Geroscience Research.

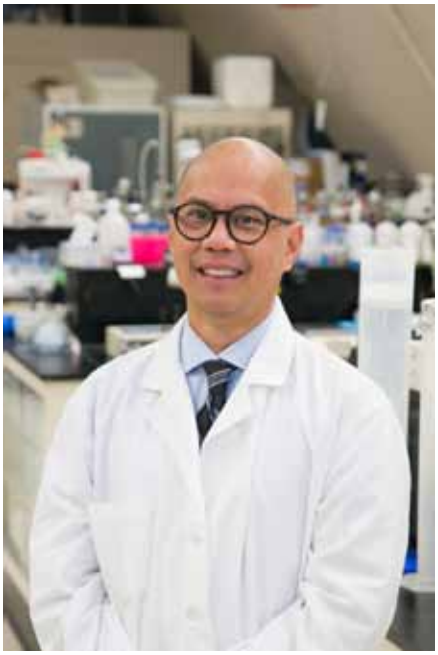
Dr. Ables is currently investigating the effects of sulfur amino acid restriction (SAAR) on neurodegenerative diseases using a mouse model for amyotrophic lateral sclerosis (ALS, commonly known as Lou Gehrig's disease). ALS is a progressive and fatal neuromuscular disease characterized by neuroinflammation progressing to neurodegeneration. No cure for ALS has yet been identified, and the lack of proven and effective therapeutic interventions is an ongoing challenge.

It is well established that rodents on a SAA-restricted diet experience delayed onset of diseases such as obesity and diabetes as well as longer lifespan; however, the neurological effect of SAAR using a mouse disease model such as ALS has not yet been examined. To investigate SAAR in an ALS model, the Ables lab is conducting experiments on transgenic mice transfected with the G93A human superoxide dismutase 1 mutation, SOD1-G93A. This mutation is associated with ALS in human beings. Feeding these mice a high-fat diet, the laboratory examines how SAAR affects skeletal muscle energy metabolism. Senior Research Associate Diana Cooke regularly conducts regular physical assessments of the mice during disease progression, sample processing, and data analysis.

Dr. Ables also continues to examine the underlying mechanisms by which SAAR promotes healthy aging, seeking to identify biomarkers that are affected by SAAR and to examine various pathways in adipose tissues in obese SAAR mice. To pursue these research interests, his team is collaborating with Drs. Calvin Vary, Robert Koza, Rea Anunciado-Koza, and Lucy Liaw of MaineHealth Institute for Research. In collaboration with Dr. Mark Horowitz of Yale University, he is examining the mechanisms by which bone marrow adipocyte accumulates in SAAR mice. He is engaged in additional studies involving SAAR with Dr. Andrey Parkhitko of the University of Pittsburgh.

In the immediate future, the Ables lab plans to examine the effects of SAAR conditions in a mouse model of Parkinson's disease as well as in human primary skin cells.

As part of the Balik Scientist Program of the Philippines Department of Science and Technology, Dr. Ables was a visiting scientist at the University of Philippines Institute of Biology from September 11–29, 2023, hosted by Michael Velarde. The Program aims to reverse brain drain from the Philippines and to promote STEM research in the country. Dr. Ables conducted seminars and workshops on the use of mouse models to study the effects of diet on aging and metabolism, sharing his expertise with faculty and students of biology and veterinary medical schools.



Gene Ables, Ph.D.
Associate Research Scientist

Johnson Laboratory

A sustained state of SAAR dramatically extends the healthy lifespan of several model organisms (Ables & Johnson, 2017 Exp Gerontol 94: 83). For example, continuously SAA-restricted rodents have less age-related pathology and are up to 45% longer-lived than their control-fed counterparts. Given that the vegan diet is low in both total protein and free amino acids, SAAR is technically feasible for humans. Indeed, recent studies have suggested that the pathways underlying the benefits of SAAR are conserved and that humans are likely to benefit similarly to rodents from this intervention. However, translating SAAR to humans has proven difficult owing to the challenges of designing a reduced SAA human diet that is both palatable and nutritionally adequate. As a result, widespread adherence to this regimen is likely to be problematic. Accordingly, a key goal of the aging field has been to develop interventions that produce health benefits similar to those engendered by SAAR, but are more practicable.

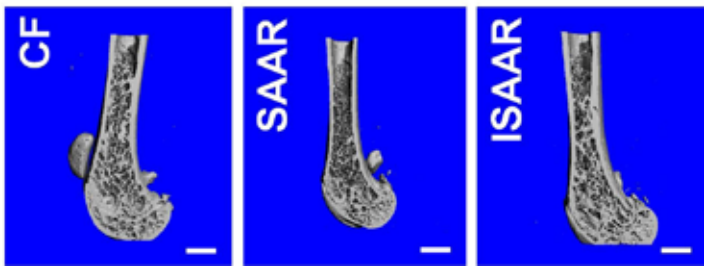
As part of this effort, Dr. Johnson's research has focused on 1) characterizing the mechanisms underlying the benefits of SAAR, and 2) identifying novel SAAR-like interventions that promote healthy aging in mammals.

Recently, Dr. Johnson developed a novel form of SAAR, intermittent SAAR, that is both highly effective and also free from the disadvantages of the classical intervention (*Aging Cell*, 2022; 21: e13629). Intermittent SAAR requires only 3 days per week of reduced SAA intake, yet improves glucose metabolism and insulin sensitivity, prevents fatty liver disease, and completely protects mice against diet-induced obesity. Similar to the continuous intervention, intermittent SAAR also confers beneficial changes in the levels of multiple hormones involved in the regulation of metabolism, health, and longevity. However, a notable difference between the two interventions is that, in contrast to classical SAAR, the novel intervention results in little to no growth inhibition and does not negatively impact the development of lean body mass.

In the past year, Dr. Johnson's group has finalized a study (performed in collaboration with Dr. Mark Horowitz, Yale University School of Medicine) exploring the effects of both continuous and intermittent SAAR on the bones of mice. A number of studies have demonstrated that continuous SAAR has deleterious effects on the mouse bones, including loss of both mass and mineral density. This intervention also has a tendency to increase the amount of marrow fat. In view of Dr. Johnson's group's observation that intermittent SAAR preserves lean body mass and causes relatively little growth inhibition as compared with the classical intervention, they hypothesized that mice undergoing intermittent SAAR might also retain more bone mass. Indeed, as compared with continuous SAAR, the intermittent intervention results in a preservation of bone mass (Figure 1), as well as a dramatic reduction in the accumulation of bone marrow fat (Figure 2). Consistent with such findings, mechanical testing revealed that the bones of intermittently SAA-restricted mice are significantly stronger than those of mice subjected to continuous SAAR. The above findings demonstrate that the more practicable intermittent form of SAAR not only confers similar metabolic health benefits to continuous SAAR, but does so without markedly deleterious effects on either the amount or strength of bone. These data thus

Jay Johnson, Ph.D.
Associate Research Scientist

Figure 1. Intermittent SAAR Preserves More Bone Mass than Continuous SAAR



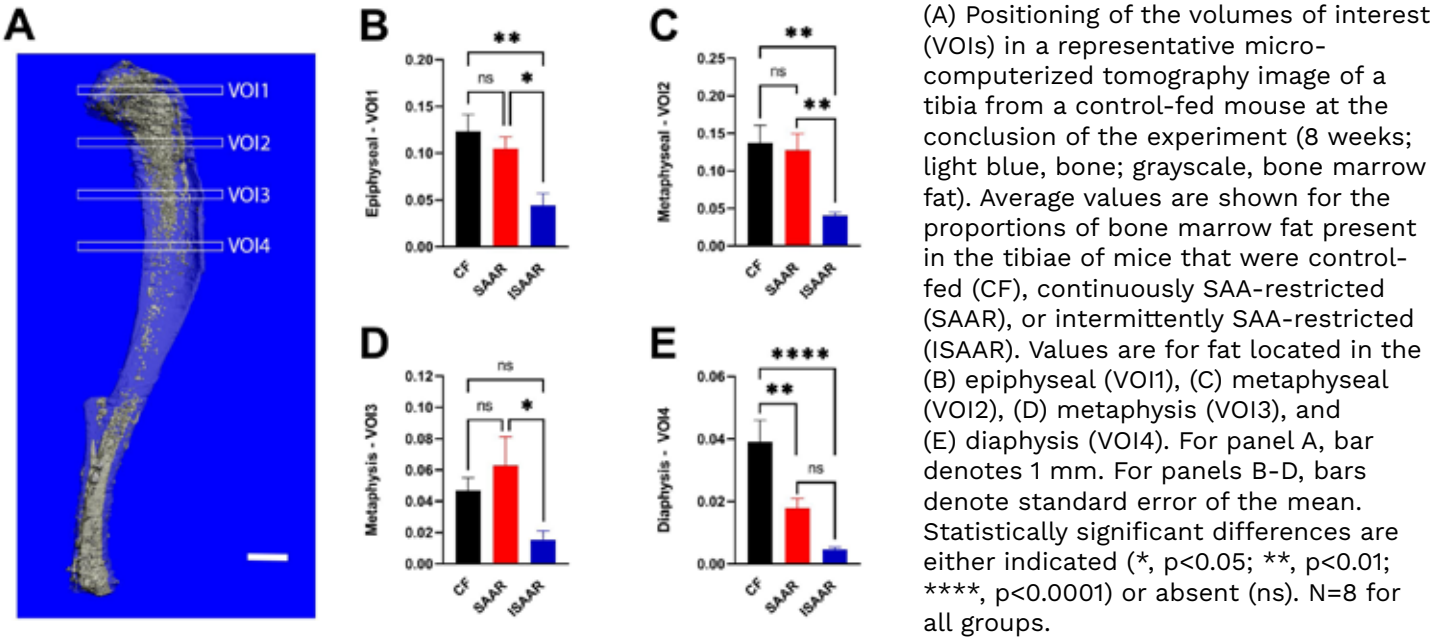
Representative micro-computerized tomography images depicting bone mass in femurs harvested from control-fed (CF), continuously SAA-restricted (SAAR), or intermittently SAA-restricted (ISAAR) mice at the experiment's conclusion (8 weeks). Bars denote 1 mm.

support the use of intermittent SAAR (rather than continuous SAAR) in humans.

Dr. Johnson has also identified several compounds that produce the health benefits associated with both forms of SAAR, but in the context of a normal (i.e., continuously SAA-replete) diet. Similar to both continuous and intermittent SAAR, supplementation with these compounds protects mice against obesity and confers beneficial changes in the levels of factors that regulate metabolism, health, and longevity. Current work in the Johnson laboratory is focused on elucidating the mechanisms underlying the health benefits of these compounds.

It is Dr. Johnson's hope that some (if not all) of these exciting new interventions might soon be translated to humans in order to improve health and reduce the burden of age-related disease.

Figure 2. Intermittent SAAR Prevents Bone Marrow Fat Accumulation



(A) Positioning of the volumes of interest (VOIs) in a representative micro-computerized tomography image of a tibia from a control-fed mouse at the conclusion of the experiment (8 weeks; light blue, bone; grayscale, bone marrow fat). Average values are shown for the proportions of bone marrow fat present in the tibiae of mice that were control-fed (CF), continuously SAA-restricted (SAAR), or intermittently SAA-restricted (ISAAR). Values are for fat located in the (B) epiphyseal (VOI1), (C) metaphyseal (VOI2), (D) metaphysis (VOI3), and (E) diaphysis (VOI4). For panel A, bar denotes 1 mm. For panels B-D, bars denote standard error of the mean. Statistically significant differences are either indicated (*, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$) or absent (ns). N=8 for all groups.

Publications

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